Acetaldehyde, Ethanol, and Carbohydrate Concentrations in Developing Muskmelon Fruit (*Cucumis melo* L. cv. Andesu) Are Affected by Short-term Shading

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Abstract. Acetaldehyde and ethanol production by muskmelon fruit were promoted by short-term shading of the plants for 5 days from 10 to 15 days prior to fruit maturation. Sucrose concentrations in the fruit flesh were reduced by shading, while fructose and glucose concentrations did not differ. Shading also accelerated the development of a “water-soaked” appearance in the flesh.

Sugar concentration is important in determining the quality of muskmelon fruit. Although melon leaves mainly transport galactosylsucroses like raffinose and stachyose (Holthaus and Schmitz, 1991; Mallick and Masui, 1986; Mitchell et al., 1992; Schaffer et al., 1987), sucrose is the predominant soluble sugar in the mature flesh (Hubbard et al., 1989; Hubbard and Pharr, 1990; McCollum et al., 1988; Schaffer et al., 1987). Weather conditions during the 2 weeks preceding fruit harvest may greatly affect the sweetness of the melon flesh because sucrose concentrations rise dramatically during this period (Hubbard et al., 1989; Hubbard and Pharr, 1990; McCollum et al., 1988; Schaffer et al., 1987). Alcohols are also important in determining quality (Motomura, 1994). Wang et al. (1996) reported that ethanol (EtOH) occurred in the flesh of immature fruit of ‘Makdimon’ muskmelon throughout maturation, and the concentration increased rapidly during the 2 weeks prior to maturation.

In the fruit of ‘Prince’ melon plants grafted onto squash (*Cucurbita* sp.), a physiological disorder, characterized by early overripening, flesh softening, and high acetaldehyde (AA) and EtOH production, has been reported, es-

pecially when the summer is cold (Hashinaga et al., 1984; Kubo, 1986; Wagatsuma and Oshima, 1981). Alcohol flavor has been used to establish the occurrence of this physiological disorder because it is difficult to diagnose from the external appearance.

The ‘Andesu’ muskmelon is widely grown in Japan. The fruit is commonly harvested 45 to 50 d after pollination (DAP) in a summer climate. Nakanishi et al. (1992) reported that translucent and overripe flesh, similar to symptoms observed in ‘Prince’ melon fruit, was also observed in ‘Andesu’ fruit, and they termed this physiological disorder “water-soaked symptom” or “urumika.” The relationship between alcohol fermentation and the occurrence of “water-soaked symptom,” however, has not been well established, since ‘Andesu’ has no alcohol flavor.

Photosynthetically active radiation on the outer surface of the leaf canopy often decreases by 50% under cloudy conditions (Nilsen and Orcutt, 1996). Nakanishi et al. (1992) also reported that the “water-soaked symptom” occurred in ‘Andesu’ melon fruit when the plants were shaded ≈50% during the 10 d prior to fruit harvest, suggesting that lower light intensity during fruit development might be a factor. The purpose of our study was, therefore, to investigate the effects of short-term shading on the changes in AA, EtOH and soluble sugar concentrations associated with the occurrence of “water-soaked symptom” in developing ‘Andesu’ muskmelon fruit.

Materials and Methods

Plant materials and cultural methods. ‘Andesu’ muskmelon plants were grown on

the farm of the Yamagata Sand Dune Experimental Station at a spacing of 2.7×0.8 m (4630 plants/ha). A slow-release fertilizer (Shonai Melon Ace; Zennoh Co-op, Tokyo) was added before transplanting to supply 100, 166, and 133 kg·ha⁻¹ of N, P, and K, respectively. Seeds were sown on 2 Apr. 1995, and the seedlings were transplanted on 2 May. The experiment was arranged in a randomized complete-block design with five replications. For 5 d, from 36 to 40 DAP (17–21 July), half the plants were covered with a net (Silver Tough-bell 4800S; Kanebo, Tokyo) that reduced daylight ≈50%. Five fruit were harvested on 16 July (35 DAP), and five fruit each from shaded and unshaded plants at 40 (developing phase), 45, 50 (maturing phase), and 60 DAP (overripe phase). A 2-cm-thick slice of flesh was taken from the middle of each fruit along the longitudinal axis and plugs were removed with a 0.5-cm-diameter cork borer from the central mesocarp (CM) and the placenta (PL) at the equator of each fruit. The netted exocarp and seeds were removed and discarded. Flesh samples of 5 g each from CM and PL were lyophilized at –50 °C and ground. A 100-mg sample of the powder was extracted three times in 80% boiling EtOH. The homogenates were centrifuged at 2400 g_n after each extraction. The EtOH extracts were evaporated to dryness, and the residues dissolved in 50 mL of water. Glucose, fructose, and sucrose concentrations in a 100-μL aliquot of the supernatant were determined with a spectrophotometer (Model UV-1200; Shimadzu Co., Kyoto) as described previously (Nishizawa and Shishido, 1998).

Concentrations of AA and EtOH were analyzed using the technique of Sugiura and Tomana (1983). Flesh samples of 5 g each from CM and PL were sealed in 20-mL screw-capped vials containing 10 mL of pure acetone (Wako Pure Chemical Co., Ltd., Osaka), subjected to ultrasonic waves for 1 min, and then stored at –25 °C for 1 week. Two-μL aliquots of acetone extract were injected into a gas chromatograph (Model GC-6AM; Shimadzu Co.), equipped with a flame ionization detector. Samples were separated on a glass capillary column (1 m × 3 mm i.d.) containing 50/80 mesh Porapak Q (Waters, Milford, Mass). Injector, column, and detector were kept at 160, 125, and 160 °C, respectively. The carrier gas was N₂ at a flow rate of 50 mL·min⁻¹. The components were identified by comparison of retention times with those of known standards.

Evaluation of “water-soaked symptom.” “Water-soaked symptom” in ‘Andesu’ melon fruit is characterized by an alteration of the flesh to translucent, and the symptom is first observed along the edge of the PL and then rapidly expands to the mesocarp as fruit matures (Nakanishi et al., 1992). Therefore, the width of the altered mesocarp flesh was measured at the equator of the fruit from the edge of the PL and used for the evaluation of “water-soaked symptom.”

Data analysis. Data were analyzed using SAS (SAS Institute, Cary, N.C.) and significant differences between treatments were tested by a paired *t* test.

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Results

Fresh mass of the fruit increased $\approx 45\%$ during the experimental period, irrespective of treatment, with no significant differences between treatments at any time (data not shown).

“Water-soaked symptom” increased as the fruit matured, irrespective of treatment, but shading significantly increased its width in the flesh at 45 and 50 DAP (Fig. 1).

Concentrations of AA in both CM and PL increased rapidly during the experimental period, irrespective of treatment (Fig. 2). Shading increased values in PL only at 45 DAP. Concentrations of EtOH increased more rapidly than did those of AA, especially in the PL, and were increased at 45 and 50 DAP by shading.

Glucose and fructose concentrations decreased slightly, irrespective of the part of the flesh, and shading had no significant effect throughout the experimental period (data not shown). Concentrations of sucrose, however, were much higher than those of reducing sugars and increased rapidly during the experimental period (Fig. 3). Shading significantly reduced the values at 50 DAP.

Discussion

Ethanol is the temporary end metabolite produced in anaerobic respiration (Chang et al., 1982). In our study, AA and EtOH concentrations in the fruit flesh were increased by shading, especially at fruit maturation (45 to 50 DAP) (Fig. 2). Some of the oxygen requirements of the plant can be supplied by photosynthesis (Vartapetian et al., 1997), suggesting that anaerobic respiration might be accelerated by shading during fruit development. Concentrations of EtOH were higher in PL than in CM (Fig. 2), suggesting that anaerobic activities might be greater in inner tissues (Motomura, 1994).

Sucrose concentrations increased rapidly (Fig. 3), while the typical increase in hexose concentration was not observed. This is probably due to the increase in the enzymatic capacity for sucrose synthesis with a concomitant decrease in the capacity for sucrose degradation (Hubbard and Pharr, 1990; Ranwala et al., 1991). Hubbard et al. (1989) also reported that activity of sucrose phosphate synthase (SPS) exceeded that of acid invertase prior to the rapid accumulation of sucrose that occurs in developing melon fruit.

Melon fruit must obtain photoassimilates from the leaves for sugar accumulation during fruit development, since they lack starch reserves (Hubbard et al., 1989; Hubbard and Pharr, 1990; Pratt et al., 1977). Although fresh mass of the fruit did not significantly differ between treatments throughout the experimental period, sucrose accumulation at fruit maturation (50 DAP) was inhibited by shading (Fig. 3). Such inhibition might be due to the shortage of photoassimilate supplied by source leaves of shaded plants. Hubbard and Pharr (1990), however, reported that SPS activity in developing melon fruit declined with partial

Fig. 1. Effects of shading for 5 d on “water-soaked symptom” in the flesh of ‘Andesu’ muskmelons. *Difference significant at $P \leq 0.05$; all other differences nonsignificant.

Fig. 2. Effects of shading for 5 d on acetaldehyde (A, B) and ethanol (C, D) concentrations in the flesh of ‘Andesu’ muskmelons. *, **, ***Difference significant at $P \leq 0.05$, 0.01, or 0.001, respectively; all other differences nonsignificant.

defoliation. This suggests that the inhibition of sucrose accumulation caused by shading might be due not only to the decreased export of photoassimilates by leaves but also to decreased SPS activity in the flesh. Effects of shading on subsequent carbohydrate accumulation and activity of sucrose-metabolizing

enzymes in the developing melon fruit need further study.

In our experiments, short-term shading accelerated the occurrence of “water-soaked symptom” (Fig. 1), while inhibiting sucrose accumulation (Fig. 3). These findings suggest that the shortage of photoassimilates might be

Fig. 3. Effects of shading for 5 d on sucrose concentrations in the central mesocarp (A) and placenta (B) of 'Andesu' muskmelons. *Difference significant at $P \leq 0.05$; all other differences nonsignificant.

a factor in inducing this disorder. Hubbard et al. (1990), however, did not report the occurrence of any physiological disorders when 50% of the leaves of 'Noy Yizreel' muskmelon were removed, although dry mass and sucrose concentrations decreased by $\approx 30\%$ and $\approx 20\%$, respectively. Recently, Aikawa et al. (1997) also reported that the fresh mass of 'Andesu' muskmelon fruit decreased by 12% when half of the leaves were removed, but "water-soaked symptom" was not observed. Therefore, the occurrence of "water-soaked symptom" in 'Andesu' muskmelon fruit may result not only from a shortage of photoassimilates but also from other processes promoted by shade. Bernadac et al. (1996) termed a physiological disorder in mature melon fruit "watercore symptom," and they suggested that it might result from calcium deficiency. "Watercore symptom" is characterized by dark, glassy, and early overmature flesh (Bernadac et al., 1996), symptoms similar to those observed in "water-soaked symptom" in 'Andesu' melon fruit (Nakanishi et al., 1992). Therefore, effects of shading on cell wall substances of melon should be clarified in further experiments.

Our results strongly suggest that limited solar radiation during the development of 'Andesu' muskmelon fruit might induce early maturity, low sucrose concentrations, and high AA and EtOH concentrations. Most 'Andesu' muskmelon in Japan are grown under plastic film, and the irradiation level during the 15 d

prior to fruit harvest (17–31 July) in the plastic film greenhouse used in our experiment was $\approx 60\%$ of that outside (9.3 vs. $14.8 \text{ MJ} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) (data not shown). Therefore, a decrease in translucency of the plastic film could also accelerate the rapid decline in the fruit quality and the occurrence of "water-soaked symptom."

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